Walk, Roger A.

From: Walk, Roger A.
Sent: Thursday, February 20, 2003 10:13 AM
To: Roethig, Hans; Haussmann, Hans-Juergen; Sarkar, Mohamadi; Tricker, Anthony; Sanders, Edward
Subject: FW: update on blood banking; meg digest: February 19, 2003

-----Original Message-----
From: The MEG/AACR Listserv digest [mailto:meg@listserv.aacr.org]
Sent: Thursday, February 20, 2003 12:00 AM
To: meg digest recipients
Subject: meg digest: February 19, 2003

MEG Digest for Wednesday, February 19, 2003.

1. RE: blood collection for banking
2. more website materials needed
3. Fwd: Re: blood collection for banking
4. RE: meg digest: February 17, 2003

Subject: RE: blood collection for banking
From: Tom London <WT_London@fccc.edu>
Date: Wed, 19 Feb 2003 07:36:26 -0500
X-Message-Number: 1

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John,
I worked in a lab at the Blood Transfusion Center of the Netherlands Red Cross in 1971-72!
They were using straws stored in liquid nitrogen to preserve PBMC and RBC.
Not such a new technology.
-Tom London

Subject: more website materials needed
From: John Wise <John.Wise@usm.maine.edu>
Date: Wed, 19 Feb 2003 07:36:33 -0500
X-Message-Number: 2

More website info sought:

Additional protocols we would like to add to the website are protocols for:

blood collection for banking (i.e., which tubes used for banking what blood components for possible assays)
DNA extraction
RNA extraction
tissue arrays

We would also like to have links to gene databases such as N-acetyltransferase gene database.

Please forward any of these types of materials that you are willing to share to me at : John.Wise@usm.maine.edu

PM3001137126

Source: https://www.industrydocuments.ucsf.edu/docs/gfhk0001

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Subject: Fwd: Re: blood collection for banking
From: Krystyna Frenkel <krystyna.frenkel@nyu.edu>
Date: Wed, 19 Feb 2003 07:36:39 -0500
X-Message-Number: 3

Dear Christine,

You are absolutely right -- these are critical issues. I know that Malmo Diet & Cancer Study (Malmo, Sweden) has published on their approach and followed up a few years later with an evaluation of how this has worked out. However, they were interested mostly in preserving cells for subsequent culturing.

In my view, the speed of processing is a crucial one. Some blood probably should be collected in tubes without an anticoagulant, which would give you serum and blood clot -- we have a method of isolating DNA from the blood clot; there also is a good commercially available kit. The main amount of blood should be collected in tubes containing EDTA or citrate (I would not collect in heparin --we had some problems later on) as anticoagulants. Tubes should be mixed several times right after blood collection & put on ice right away. All processing should not take longer than two hours before storing the specimens in a freezer.

Yu can use Histopague (Sigma/Aldrich) for rapid separation of monocytes from granulocytes and red blood cells (check their website). Even though RBCs & PMNs are in the same fraction, DNA can be also isolated from this mixture; I do not know how this would affect RBC enzymes. I would think that some labs would want to study white blood cells devoid of PMNs.

Now, an issue of the storage. The best would be to keep the air out of the tubes, which can be accomplished by using argon, which is heavier than oxygen & thus would displace it. Tubes to be used should have silicone gaskets to prevent gas escaping at low temperature of the storage at -80 C.

I would also add that processing of cells for DNA isolation should take into account that, upon lysis, iron becomes more bioavailable and released DNA is subjected to spurious oxidation. For example, dG is very easily oxidized to 8-oxo-dG, which is often used as a biomarker of exposures to environmental, lifestyle, or dietary factors. Even if one is not studying 8-oxo-dG per se, its presence in DNA could affect the PCR, since 8-oxo-dG and some other oxidized bases, which are likely present in such DNA, can interfere with incorporation of proper trinucleotides. If you are interested, I can provide the method developed in our lab that minimizes spurious oxidation of DNA during its isolation & processing, which has been validated over a period of two years already and we continue to check it for its reliability.

With best regards,

Krystyna

Krystyna Frenkel, Ph.D.

PM3001137127

Source: https://www.industrydocuments.ucsf.edu/docs/gfhk0001

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Subject: RE: meg digest: February 17, 2003
From: "Chen, Chu" <cchen@fhcrc.org>
Date: Wed, 19 Feb 2003 07:36:45 -0500
X-Message-Number: 4

Our experience indicates that a low speed centrifugation to separate plasma from peripheral blood cells is inadequate to flow all white cells to the interface, the so called buffy coat layer. Many of the white cells remain in the bottom half of the cellular layer (the bottom 2 mL). Because of this finding, when we don't need to store red cells, we lyse all the red cells to prepare a buffy coat that includes all white cells tha was collected in a blood vacutainer. By doing so, we routinely obtain 150 to 250 micrograms of DNA from a tube of 10 mL blood, and some tubes yielded much more than that. For maximum yield of white cells and red cells, a ficol gradient will help. However, all the protocols employing a ficol gradient that I am aware of will sacrifice the plasma.

When we want to store plasma, white blood cells and red blood cells, our choice of vacutainer has been the EDTA tube (purple). Plasma from ACD tube is too acidic for the analyses of many serum proteins.

We have mixed results using blood cards. While we were able to extract DNA from blood spotted on filter papers that were stored in sealed plastic bags at -70 degree C, the quality of DNA was not always good enough for PCR amplification of large DNA fragments.

It's great to learn that Dr. Landi at NCI has had good experience with the PAXgene RNA tubes so far. However, the cost for the PAXgene tubes and associated processing supply at the present time is pretty prohibitive, if the blood collection is to be done on a large number of study subjects.

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